Crystal structure of nitric oxide synthase bound to nitroindazole reveals a novel inactivation mechanism

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Nitric oxide is generated under normal and pathophysiological conditions by three distinct isoforms of nitric oxide synthase (NOS). A small-molecule inhibitor of NOS (3-Br-7-nitroindazole, 7-NIBr) is profoundly neuroprotective in mouse models of stroke and Parkinson's disease. We report the crystal structure of the catalytic heme domain of endothelial NOS complexed with 7-NIBr at 1.65 Å resolution. We also present two crystal structures of eNOS complexed with either 7-nitroindazole-2-carboxamidiine or N-(4-chlorophenyl)-N'-hydroxyguanidine that reveal how alterations at the substrate site facilitate 7-NIBr and structurally dissimilar ligands to occupy the cofactor site. The x-ray diffraction data reported here were in part collected at Beamline 5.0.2 at ALS. Critical to the binding of 7-NIBr at the substrate site is the adoption by eNOS of an alternate conformation, in which a key substrate binding residue, Glu-363, swings out toward one of the heme propionate groups. Perturbation of the heme propionate ensues and eliminates the cofactor tetrahydrobiopterin-heme interaction. In fact, 7-NIBr selects for the catalytically incompetent Glu-363 rotamer and by binding to the substrate site locks this conformation in place. By competing simultaneously for both the substrate and cofactor binding sites, 7-NIBr is able to occupy one site and subsequently alter the specificity of a second site. Structural analyses of the 7-NIBr-bound eNOS structure teaches us that designing an inhibitor, which avoids H-bonded contact with one of the heme propionates and concurrently selects for the alternate Glu-363 rotamer, can serve as a potential template for designing drugs with isoform specificity. This is because such compounds dramatically weaken the affinity of the cofactor site for H_4B and subsequently make it promiscuous. Therefore, one can take advantage of the small but significant differences at the substrate and cofactor binding sites toward designing bifunctional drugs with isoform selectivity.

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